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B. Novak

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16. Abstract

The effect of increasing additions of glucose (10 mg% to 1,000 mg%) and of ammonium sulfate (10 mg% to 50 mg%) to soil was examined in model experiments. Carbon dioxide production after 20 hours incubation was measured to test the respiratory activity.

Respiration and substrate mineralization increased with increasing concentrations of glucose and nitrogen in soil. The percentage mineralization of the added substrate increased with increasing N concentration and decreased with increasing C concentration.

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RESPIRATION OF THE SOIL AFTER ADDITION OF GLUCOSE AND INORGANIC NITROGEN

Bohumir Novak

Glucose metabolism has been very well studied, so that glucose is used as a model substrate not only in general microbiology but also in specialized fields. Glucose is also used quite often in soil microbiology (Domsch 1965; Drobnik 1958; Kas 1942; Macura et al 1964; Novak 1965, and many others). As a soil additive, glucose quickly produces extensive changes in the number, the composition, and in the metabolic activity of the microflora (Freytag and Igal 1964; Macura et al 1963). Drobnikova and Drobnik (1965) characterize this as reactive respiration. /71 *

In our previous works we have recommended that not only the basal respiration but also the respiration after addition of glucose or nitrogen and after a combined addition of glucose and nitrogen be determined as part of the respirometric tests (Novak 1965; Novak and Apfelthaler 1964). This work has the goal of explaining in more detail the effect of various concentrations of added glucose and inorganic nitrogen on the soil respiration.

Materials and Methods

For the experiments we used garden soil obtained from the humus of the weakly decomposed black earth in Ruzyne. The garden soil was dried in air. Before the actual incubation it was moistened to 20% water content and then held at a temperature of +2°C to +4°C so that the water in the sample would be evenly distributed.

* Numbers in the margin indicate pagination in the original foreign text.

Before the incubation proper, 2 ml of nutrient solution was added to every 50 g of dry substance. The solution contained glucose or ammonium sulfate. The controls were moistened with the same volume of water. The amounts of C and N added can be found in Table 1.

The fifty-gram samples (recalculated to dry material) were placed in thin layers on the bottom of 500 ml Erlenmeyer flasks and left in the incubator for 20 hours at 27°C. After the end / 72
of the incubation, the amount of CO_2 produced was measured interoferometrically (Novak and Apfelthaler 1964).

Table 1. RESPIRATION OF SOIL SAMPLES ENRICHED WITH GLUCOSE AND $(NH_4)_2SO_4$, EXPRESSED AS MEAN PRODUCTION OF CO_2/100g/hr IN THE COURSE OF A 20-HOUR INCUBATION.

Addition C in %	Addition of NH_4^+- N in mg%			
	0	10	30	50
0,00	0,68	0,69	0.72	0.71
0,01	0,92	2,15	2.36	2.39
0,05	1,38	4.02	4,73	5,23
0,1	2,16	5,68	7.66	8,33
0,2	3,68	6,97	12,32	14,28
0,3	4.42	7,32	17,08	20,90
0,5	5,03	7,94	29.67	38.41
1,00	4,62	8,47	34.20	42,16

The garden soil used (control after the end of incubation) can be characterized as follows:

C_{ox}	1,48%	NO_3^-—N	1.2 mg %
N (Kjeldahl)	0,135%	pH (H_2O)	6,9
NH_4^+ —N	11,6 mg/%	pH (KCl)	6,8.

Results and Discussion

The basic data from the test results are summarized in Table 1 and shown in Figures 1-4.

The lowest respiration was measured for the garden soil which received no supplement, and the greatest with an addition of 1% C in the form of glucose and 50 mg% N as ammonium sulfate. Except for insignificant exceptions, increased concentration of glucose and of ammonium nitrogen in the soil produced increased respiration.

If there was no addition of nitrogen, the respiration increased, through the effect of the added glucose, to a maximum of 7.5 times the basal respiration. The highest respiration was attained with addition of 500 mg% C as glucose. In all the other variations, the maximum respiration was achieved at the highest dose of added glucose (1% C). At an addition of 10 mg% ammonium nitrogen, the maximum respiration activity was 12 times higher than the respiration at the same C addition without addition of N. In the series of variations with an addition of 30 mg%, the highest respiration of the soil (supplemented with 1% C) was 48 times higher than the respiration of the soil without added carbon. In the series of variants with an addition of 50 mg% N, the difference between similar variants increased to 60-fold.

The effect of the added nitrogen is also dependent on /74
the concentration of the easily usable carbon source. In the series without added glucose, there was only a very indistinct effect of nitrogen addition. In this series, the maximum respiration was reached at an addition of 30 mg% N. But this maximum was only 6% higher than the basal respiration. In all the other series, the highest respiration was attained at maximum addition of nitrogen (50 mg%). In each series, the /75

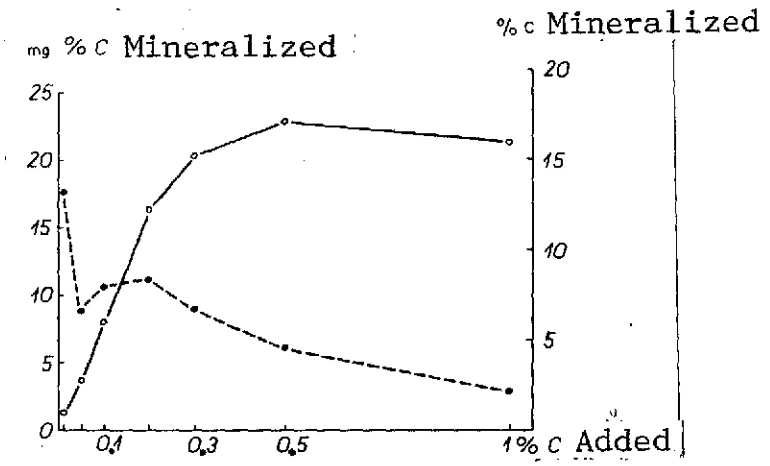

Figure 1. Effect of increased glucose concentration without addition of N on its degradation in the soil.
○——○ Total mineralization of the substrate (in mg% C).
●········● % of the mineralized substrate

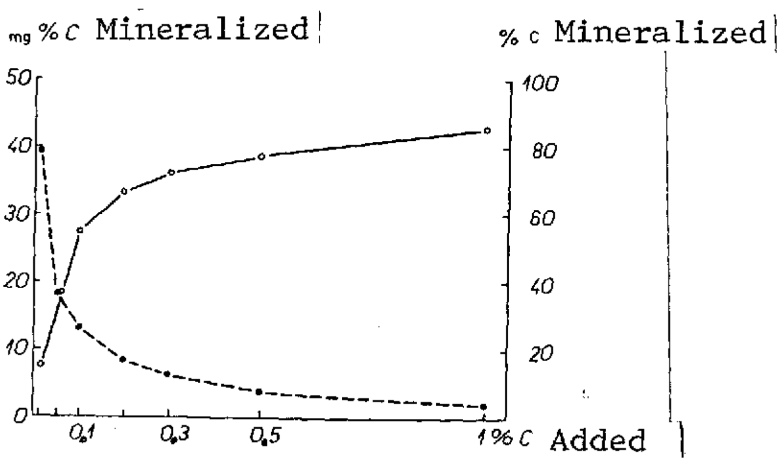

Figure 2. Effect of increased glucose concentration with simultaneous addition of 10 mg% N (as $(NH_4)_2SO_4$) on substrate degradation.

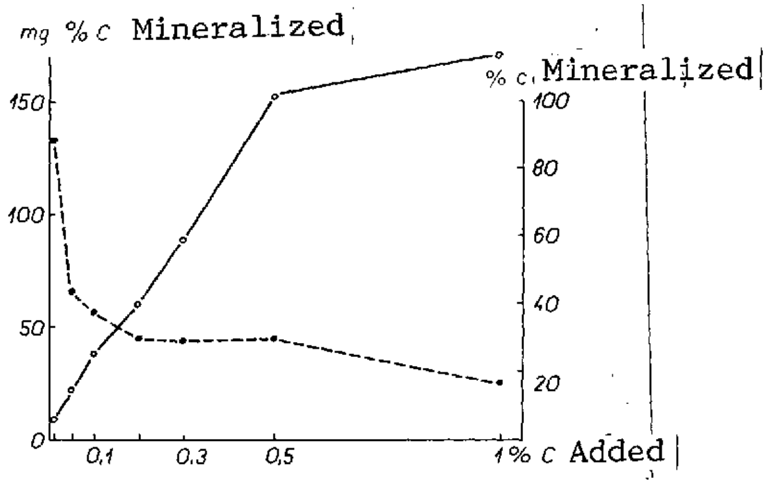

Figure 3. Effect of increased glucose concentration with simultaneous addition of 30 mg% N (as $(NH_4)_2SO_4$) on substrate degradation.

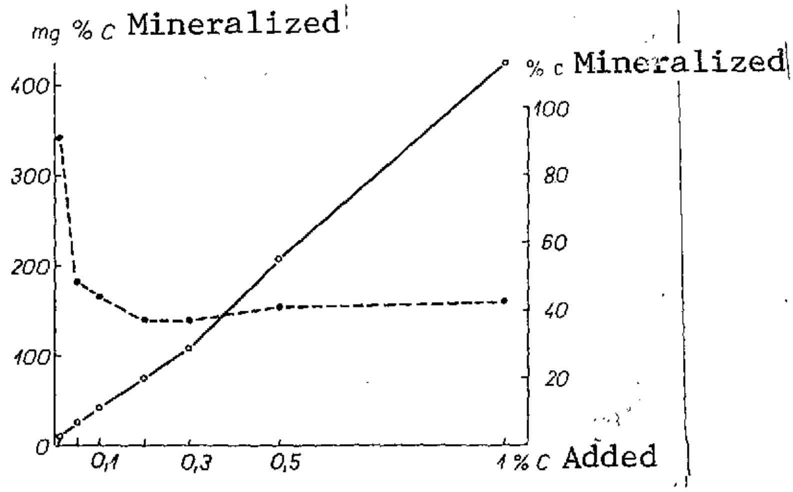

Figure 4. Effect of increased glucose concentration with simultaneous addition of 50 mg% N (as $(NH_4)_2SO_4$) on substrate degradation.

ratios of the maximum respiration to the respiration obtained without nitrogen addition were in the following ranges:

at 10 mg% C addition: 2.6
at 50 mg% C addition: 3.8
at 100 mg% C addition: 3.9
at 200 mg% C addition: 3.9
at 300 mg% C addition: 4.8
at 500 mg% C addition: 7.6
at 1% C addition: 9.1.

It is noteworthy that the maximum addition of N in the interval of glucose addition between 50 mg% and 200 mg% produced almost the same increase of respiration intensity, as compared to the variant with the same C but without N addition.

This apparently surprising fact can, however, be explained by the very low ratios of C:N after the addition of 50 mg% and the relatively high degree of substrate mineralization.

If we neglect the "priming effect", or if we assume that it is zero, then we can calculate the mineralization of the added substrate. Figures 1 - 4 show how the amount of mineralized substrate increases with increasing concentrations of glucose and of ammonium nitrogen. The percentage of the mineralized substrate increases with increasing N concentration and, with exceptions, decreases with increasing C concentration.

At very low glucose concentration (10 mg% C) and without addition of N the mineralization reaches 13.2%. But even the first addition of N adjusts the C:N ratio in the added substrates to 1, and three fourths of the glucose is mineralized during the incubation. On further additions of N, this proportion of mineralized substrate rises to 0.9. In the series without N addition, the percentage mineralization of the added C drops off rapidly until it reaches only 2.14% at a concentration of 1,000 mg% in the form of glucose.

Apparently the nitrogen deficiency is the limiting factor here, because the precentage of mineralized C varies by 40% in the variants with an addition of 50 mg%. At the maximum concentrations of added glucose (1% C) and of added N (50 mg%), 42.6% mineralization was attained. The C:N ratio was 20. When the C:N ratio in the added substrates rose to 33.3, only 17.2% of the substrate was mineralized at the same C concentration. And at a C:N ratio of 100, only 4.3% was mineralized, with the same amount of the original substrate.

In the soil samples with 1% C addition, the following amounts were mineralized per milligram of added nitrogen:
at an added N concentration of 10 mg%: 2.16 mg C
at an added N concentration of 30 mg%: 5.02 mg C
at an added N concentration of 50 mg%: 8.11 mg C.
Similar values were determined in the series with an addition of 500 mg% C. At a concentration of 10 mg% added N, 1.60 mg C was mineralized per milligram of added nitrogen. At a concentration of 30 mg% added N, 4.24 mg C was mineralized per milligram of added nitrogen, and 3.704 mg C at an added N concentration of 50 mg%. Therefore, when a ratio of C:N = 10 is reached in the added substrate, the amount of mineralized N per unit of added N no longer increases, but decreases somewhat.

In the other series, the amount of mineralized C per unit of added N was very small, and allowed no similar comparison.

It appears, then, as has also often been shown in practice, that a C:N ratio of about 10 is particularly important, and is a definite physiological threshold in the metabolism of the soil microflora.

In our experimental variations, the substrate was added in this ratio three times: 100 mg% C and 10 mg% N; 300 mg% C and 30 mg% N; and 500 mg% C and 50 mg% N. As expected, we /76 found increasing mineralization with increasing amount of substrate. If we subtract the basal respiration, we can calculate that 27 mg of C out of 100 mg was mineralized; 89 mg out of 300 mg; and 200 mg out of 500 mg. It is surprising that not only the absolute amount of mineralized C increases, but also the degree of mineralization of the substrates, which was 27.2% in the variation with 100 mg% C, 29.7% in the variation with 300 mg% C, and 41.4% in the variation with 500 mg% C addition.

This result was not expected. On the contrary, we assumed that with increasing substrate concentration, the degree of carbon mineralization during the incubation would decrease when a stable C:N ratio was maintained. We cannot explain the difference between the expected and the actual results on the basis of our previous findings. An explanation should probably be sought in the reaction kinetics of the respiratory processes.

Summary

In model experiments we followed the effect of stepped additions of glucose (from 10 mg% to 1,000 mg%) and stepped additions of ammonium sulfate (from 10 mg% to 50 mg%) to garden soil on the respiratory activity of the microflora and the intensity of mineralization of the added substrate.

The respiration and total mineralization of the substrate rose with increasing concentration of glucose and nitrogen in the soil sample. The proportion of mineralized substrate, expressed in percent of the amount of substrate initially present in the soil, increased with increasing N concentration and decreased with increasing C concentration.

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